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(51) International Patent Classification 5: (11) International Publication Number: WO 93/02360 À1 G01N 33/536, 33/546 (43) International Publication Date: 4 February 1993 (04.02.93) (72) Inventors; and (75) Inventors/Applicants (for US only): LEHNEN, Brian, C. [US/US]; 2640 Howard Avenue, San Carlos, CA 94070 (US). CROTHERS, Stephan, D. [US/US]; 3012 Ross Road, Palo Alto, CA 94303 (US). PCT/US92/05799 (21) International Application Number: 10 July 1992 (10.07.92) (22) International Filing Date: (30) Priority data: 731,039 16 July 1991 (16.07.91) US (74) Agents: RAE-VENTER, Barbara et al.; Cooley Godward Castro Huddleson & Tatum, Five Palo Alto Square, 4th Floor, Palo Alto, CA 94306 (US). (60) Parent Application or Grant (63) Related by Continuation US (81) Designated States: AU, CA, JP, NO, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE). 731,039 (CIP) Filed on 16 July 1991 (16.07.91) (71) Applicant (for all designated States except US): TRANS-MED BIOTECH INCORPORATED [US/US]; 120 South Linden Avenue, South San Francisco, CA 94080 Published With international search report.

## (54) Title: METHODS AND COMPOSITIONS FOR SIMULTANEOUS ANALYSIS OF MULTIPLE ANALYTES

#### (57) Abstract

A method for detecting multiple analytes of interest in a sample employing a complementary binding moiety to each of said analytes bound to a solid support, wherein each analyte and its complementary binding moiety comprise first and second members of a specific binding pair (msbp) respectively is provided. The method includes the steps of forming a mixture of known proportions of multiple subpopulations of said complementary binding moieties, wherein each subpopulation comprises a different complementary binding moity, contacting the sample with the mixture so that specific binding pairs are formed on the solid supports, and relating the presence of analytes of interest in the sample to the formation of specific binding pairs associated with each unique proportion of said multiple subpopulations by comparing the area of the peak in the fluorescence histogram to the total area of peaks in the histogram. The method can be performed with solid supports of a single average size and a single (luorochrome and without the need for using other detection systems (fluorescence FS & SS).

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# . METHODS AND COMPOSITIONS POR SIMULTANEOUS ANALYSIS OF MULTIPLE ANALYTES

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## **CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a continuation-in-part of USSN 07/731,039 filed July 16, 1991, which disclosure is incorporated herein by reference.

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#### INTRODUCTION

#### Technical Field

The present invention relates to a method and apparatus for use therein for the contiguous detection of multiple analytes in a biological sample using multiple complementary binding moieties. The invention is exemplified by the contiguous detection of human IgG, human chorionic gonadotropin and antibodies to recombinant HIV gp41, recombinant HIV p24, Hepatitis B core protein, and recombinant HTLV re-5.

#### 20 Background

The in vitro diagnostics industry has been seeking technologies which afford the contiguous discrete detection of multiple analytes. As used in this application, the term "contiguous" refers to assays carried out both in the same space and at the same time, in contrast to the term "simultaneous", which refers to assays carried out at the same time, but not necessarily in the same space. As an example, chlamydia and gonorrhea infections often are coincident in women. Collecting a specimen can be problematic so that a single assay which can detect both disease agents contiguously is desirable. Another example is the current interest in the simultaneous detection of HIV-1 and HIV-2 antibodies in blood.